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Metabolic mapping of the rat cerebellum during delay and trace eyeblink conditioning

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Abstract

The essential neural circuitry for delay eyeblink conditioning has been largely identified, whereas much of the neural circuitry for trace conditioning has not been identified. The major difference between delay and trace conditioning is a time gap between the presentation of the conditioned stimulus (CS) and the unconditioned stimulus (US) during trace conditioning. It is this time gap or trace interval which accounts for an additional memory component in trace conditioning. Additional neural structures are also necessary for trace conditioning, including hippocampus and prefrontal cortex. This addition of forebrain structures necessary for trace but not delay conditioning suggests other brain areas become involved when a memory gap is added to the conditioning parameters. A metabolic marker of energy use, radioactively labeled glucose analog, was used to compare differences in glucose analog uptake between delay, trace, and unpaired experimental groups in order to identify new areas of involvement within the cerebellum. Known structures such as the interpositus nucleus and lobule HVI showed increased activation for both delay and trace conditioning compared to unpaired conditioning. However, there was a differential amount of activation between anterior and posterior portions of the interpositus nucleus between delay and trace, respectively. Cerebellar cortical areas including lobules IV and V of anterior lobe, Crus I, Crus II, and paramedian lobule also showed increases in activity for delay conditioning but not for trace conditioning. Delay and trace eyeblink conditioning both resulted in increased metabolic activity within the cerebellum but delay conditioning resulted in more widespread cerebellar cortical activation.

Keywords: Interpositus nucleus; 2-Deoxyglucose; Lobule HVI; Lobule HV; Cerebellar cortex

1. Introduction

Delay and trace eyeblink conditioning are very similar paradigms; wherein a conditioned stimulus (CS; 2 kHz tone) is paired with an unconditioned stimulus (US; unilateral periorbital shock). After several pairings of the tone and shock, the CS comes to elicit a conditioned response (CR), wherein the eyelid closure occurs before the onset of the US. The primary difference between delay and trace conditioning paradigms is the memory requirement. Here, we used metabolic mapping to compare the cerebellar circuitry underlying delay and trace eyeblink conditioning. No time gap exists between the CS and US in delay conditioning, whereas a time gap does exist during trace conditioning between the offset of the CS and the onset of the US. The presence of a trace interval in the conditioning paradigm necessitates the involvement of forebrain areas (Kim, Clark, & Thompson, 1995; Moyer, Deyo, & Disterhoft, 1990; Weible, McEchron, & Disterhoft, 2000; Weiss, Bouwmeester, Power, & Disterhoft, 2000). This memory trace may add another level of difficulty to the behavioral task and require additional processing by other neural structures.

The cerebellum and brainstem nuclei have been viewed as the only structures necessary for successful delay eyeblink conditioning (Christian & Thompson, 2003). Additional studies demonstrate that within the cerebellum the interpositus nucleus (IN) is the key element responsible

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for acquiring the association between stimuli (Krupa & Thompson, 1997; Steinmetz, Logue, & Steinmetz, 1992). The IN is also critical in trace conditioning (Woodruff-Pak, Lavond, & Thompson, 1985). However, trace conditioning also involves other forebrain structures such as the hippocampus and medial prefrontal cortex (Kim et al., 1995; Moyer et al., 1990; Weible et al., 2000; Weiss et al., 2000). Recently it has been argued that even for delay conditioning, there may be other structures beyond the cerebellum and brainstem that are critical for acquisition such as the medial auditory thalamus (Halverson & Freeman, 2006). Using a metabolic mapping approach may lead to identification of new cerebellar regions involved in delay and/or trace conditioning.

The lateral anterior lobe, lobule HVI, and the anterior interpositus nucleus have been specifically linked to the acquisition and retention of eyeblink conditioning (Thompson, 2005). However, there are multiple blink zones distributed across the C1 and C3 longitudinal zones of the cerebellum (Hesslow, 1994), which raises the possibility that there are other cortical areas and nuclei that play roles in eyeblink conditioning. It is also possible that different cerebellar cortical areas and nuclei contribute to delay and trace conditioning.

A marker of metabolic activity, [C^{14}]2-deoxyglucose (2-DG), was used to compare glucose analog uptake across delay, trace, and unpaired groups of male rats. The 2-DG metabolic mapping method allowed us to examine cerebellar cortex and deep nuclei to ascertain the possible subsets of structures involved in delay and trace conditioning. The primary experimental parameter that differed between the delay and trace groups was whether or not there was a time gap between the offset of the CS and onset of the US. The unpaired group received explicitly unpaired CS and US stimulus presentations. By comparing rats given unpaired presentations of the CS and US with rats given delay or trace conditioning, we investigated the possible neural circuit differences for remembering an association (between the CS and US) across a time gap versus the formation of an association.

2. Methods

2.1. Subjects

Twenty male Long Evans rats weighing 200–250 g were used for this study. All rats were housed in Spence Laboratories at the University of Iowa and kept on a 12-h light/dark cycle. The rats were fed standard rat chow ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Iowa. The rats were divided into three conditioning groups: delay, trace, and unpaired.

2.2. Surgery

For surgery, each rat was anesthetized and fitted with differential EMG (electromyogram) electrodes implanted in the left upper eyelid muscle (orbicularis oculi). A ground electrode was attached to a stainless steel skull screw. The EMG electrode leads terminated in gold pins held in a plastic connector, which was secured to the skull with dental acrylic. A bipolar stimulating electrode (for delivering the shock US) was implanted subdermally, immediately caudal to the left eye. The bipolar electrode terminated in a plastic connector that was secured to the skull with dental acrylic. Both sets of connectors for the EMG and bipolar electrode were connected to a lightweight cable that allowed the rats to move freely during conditioning. The rats were allowed to recover for 7–10 days before training began.

2.3. Conditioning apparatus

Rats from each group were trained in one of four sound attenuating chambers (BRS/LVE, Laurel, MD). These small animal sound attenuating chambers surrounded a small-animal operant chamber where the rats were kept during conditioning (BRS/LVE, Laurel, MD). One wall of the operant chamber was fitted with two speakers. The back wall of the sound attenuating chamber was equipped with a small house light and exhaust fan. The electrode leads from the rat's headstage were connected to peripheral equipment and a desktop computer. Computer software controlled the delivery of stimuli and the recording of eyelid EMG activity (JSA Designs, Raleigh, NC). The shock stimulus (1–2 mA, DC constant current) was delivered through a stimulus isolator (Model number 365A, World Precision Instruments, Sarasota FL). EMG activity was recorded differentially, filtered (500–5000 Hz), amplified (2000×) integrated by equipment (JSA Designs, Raleigh, NC) described in other reports (Nicholson, Sweet, & Freeman, 2003).

2.4. Behavioral paradigm

2.4.1. Training groups

Rats were given delay, trace or unpaired conditioning. The conditioned stimulus (CS) was a tone (2 kHz, 250 ms) paired with a unilateral periorbital shock unconditioned stimulus (US, 25 ms). For the delay group ($n = 7$), the CS terminated with the onset of the US. For the trace group ($n = 6$), the CS offset was separated by 250 ms from the US onset. The unpaired group ($n = 7$) received the CS and US in an explicitly unpaired fashion.

2.4.2. Training procedure

For delay and trace conditions, training consisted of 10 blocks of 10 trials for a total of 100 trials per session per day. Each block consisted of nine pairings of the CS and US and one CS alone presentation used as a test trial. CRs for all groups were defined as responses that crossed a threshold of 0.4 volts above baseline during the CS period after 80 ms.

Rats in all groups were trained for 4 days. Before training each day, the rats were habituated to a small containment box, where the tail was restrained. This procedure readied the rats for the injection that occurred on the last day. On the fifth and final day, rats were weighed and then given an i.v. tail injection of (20 μCi/100 g) body weight, glucose analog, 2-deoxyglucose (2-DG) radiolabeled with ^{14}C (American Radiolabeled Chemicals, St. Louis) in 0.3 ml sterile (0.9%) saline (Breier, Crane, Kennedy, & Sokoloff, 1993). Experimental rats were then run in their final training session. The computer program was altered to allow the final session to run continuously for 2 h (rather than the typical 1 h session) so that each rat could be run separately. The rats were only allowed to experience 80 trials, or about 40 min of training compared to their normal 100 daily training trials. This was done to ensure optimal brain harvest time, which occurs 45 min post injection. After 45 min rats were decapitated. The brains were extracted in less than 6 min and frozen in hexane cooled to −37 °F and then stored at −80 °F until sectioned.

2.5. Histology

Before sectioning, brains were removed from the freezer and allowed to equilibrate for 10 min. The brain was cut just anterior to the cerebellum so that the cerebellum could be sliced horizontally. The cerebellum was sectioned at 40μ in a series of 7 with 4 sections saved for 2-DG analyses and one saved for Nissl staining. Tissue mounted for 2-DG was